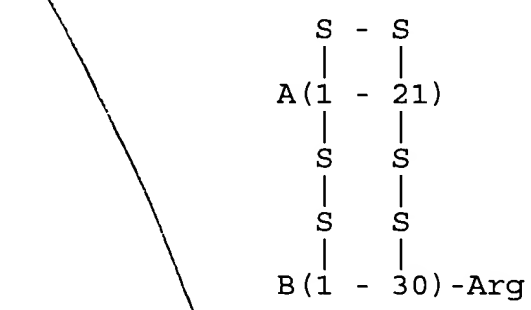
21. (Twice Amended) A method for the preparation of a mono-Arg-insulin compound of formula II



(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing as part of a fusion protein in a bacterium a DNA molecule encoding a mini-proinsulin compound of the formula:

B(1-30)-Arg-A(1-21),

[in which B(1-30) and A(1-21) denote the B and A chains of human insulin];

(b) liberating said mini-proinsulin compound from said fusion protein [to obtain said mini-proinsulin in a native conformation; and];

(c) folding and forming disulfide bridges in said mini-proinsulin compound; and

[(c)] (d) incubating said mini-proinsulin compound with trypsin at a pH of about 6.8 [under conditions where no crystals are formed].

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22. (Twice Amended) A method for the preparation of insulin which comprises:

(a) expressing as part of a fusion protein in a bacterium a DNA molecule encoding [the] a mini-proinsulin compound of the formula:

B(1-30)-Arg-A(1-21),

in which B(1-30) and A(1-21) denote the B and A chains of [human] insulin;

(b) liberating said mini-proinsulin compound from said fusion protein [to obtain said mini-proinsulin in a native conformation];

(c) folding and forming disulfide bridges in said mini-proinsulin compound; and

[(c)] (d) simultaneously incubating said mini-proinsulin compound with trypsin and carboxypeptidase B at a pH of about 6.8 [under conditions where no crystals are formed to produce a mono-Arg-insulin; and

(d) cleaving the resulting mono-Arg-insulin with carboxypeptidase B].

23. (Twice Amended) A method as claimed in claim 22, wherein [steps (c) and] step (d) [are] is carried out in one

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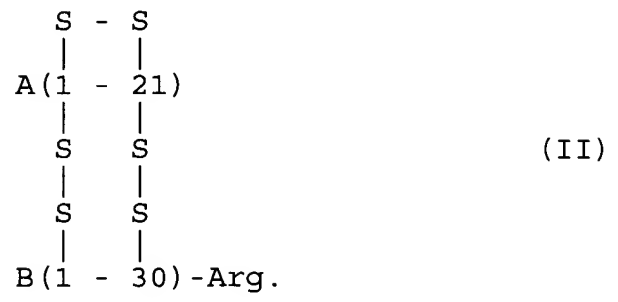
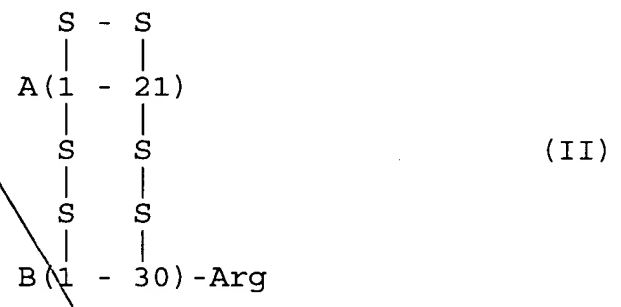
vessel without having to isolate as an intermediate mono-Arg-insulin of formula II



(II)

25. (Twice Amended) A method for the preparation of a mono-Arg-insulin compound of formula II



(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg -,

to a peptide which stabilizes the fusion protein;

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(b) liberating [said] a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide[, to obtain said mini-proinsulin in a native conformation; and];

(c) folding and forming disulfide bridges in said mini-proinsulin compound; and

[(c)] (d) incubating [the] said mini-proinsulin compound [of step (b)] with trypsin at a pH of about 6.8 [under conditions where no crystals are formed].

26. (Twice Amended) A method for the preparation of insulin which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg -,

to a peptide which stabilizes the fusion protein;

(b) liberating [said] a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide[, to obtain said mini-proinsulin in a native conformation];

(c) folding and forming disulfide bridges in said mini-proinsulin compound; and

[(c)] (d) simultaneously incubating [the] said mini-proinsulin compound [of step (b)] with trypsin and

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